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The majority of US combat casualty soft-tissue wounds are not infected or colonized upon arrival or during treatment at a continental US military medical facility

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Abstract

BACKGROUND: The microbiology of war wounds has changed as medicine and warfare have evolved. This study was designed to determine the microbial flora and bacterial quantification of present-day war wounds in US troops from Iraq and Afghanistan upon arrival at the National Naval Medical Center (NNMC).

METHODS: Patients with extremity combat wounds treated with a vacuum-assisted wound closure device were enrolled in study. Wounds were biopsied every 48 to 72 hours with quantitative microbiology performed on all biopsies.

RESULTS: Two hundred forty-two wound biopsies from 34 patients; 167 (69%) showed no growth, and 75 (31%) showed positive growth. The incidence of any bacterial isolation from biopsies weekly from the time of injury was 28% (first), 31% (second), and 37% (≥third). *Acinetobacter baumannii* was the most prevalent isolate.

CONCLUSIONS: Most soft-tissue wounds from Iraq and Afghanistan do not have significant bacterial burden upon arrival to and during initial treatment at NNMC. Improved evaluation of combat wound microbiology at all levels of care is warranted to determine shifts in microbiology and to impact care practices. Published by Elsevier Inc.

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14. ABSTRACT
BACKGROUND: The microbiology of war wounds has changed as medicine and warfare have evolved. This study was designed to detennine the microbial flora and bacterial quantification of present-day war wounds in US troops from Iraq and Afghanistan upon arrival at the National Naval Medical Center (NNMC). METHODS: Patients with extremity combat wounds treated with a vacuum-assisted wound closure device were enrolled in study. Wounds were biopsied every 48 to 72 hours with quantitative microbiology performed on all biopsies. RESULTS: Two hundred forty-two wound biopsies from 34 patients; 167 (69%) showed no growth and 75 (31%) showed positive growth. The incidence of any bacterial isolation from biopsies weekly from the time of injury was 28% (first), 31% (second), and 37% (2:third). Acinetobacter baumannii was the most prevalent isolate. CONCLUSIONS: Most soft-tissue wounds from Iraq and Afghanistan do not have significant bacterial burden upon arrival to and during initial treatment at NNMC. Improved evaluation of combat wound microbiology at all levels of care is warranted to detennine shifts in microbiology and to impact care practices.

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The US military medical system supports combat troops in Iraq and Afghanistan with advanced, state-of-the-art, therapeutics and additionally manages the transport of casualties from the battlefield to initial medical facilities (MEDEVAC) and between medical facilities (CASEVAC). A relatively small number of military medical facilities with concentrated and advanced medical assets, in conjunction with a state-of-the-art transport system, work to rapidly return casualties to continental US (CONUS) tertiary care (echelon 5) military medical centers.

To clarify, the military divides care into different echelons, with echelons I to III occurring in the theater of operations and echelons IV and V being large hospitals outside of the theater, such as Landstuhl Regional Medical Center in Germany (echelon IV) and CONUS medical centers (echelon V). Echelon I is self-aid or buddy aid given by a combatant or medic. Physicians become involved at echelons II and III. Echelon II care is limited to damage-control operations for the control of hemorrhage and contamination and limb preservation. Echelon III is typically a combat support hospital with multiple operating rooms, blood products surgical teams, and subspecialists. Within this modern US military medical paradigm, the emphasis in the combat theater is resuscitation, resuscitative (damage control) surgery, and stabilization to expedite casualty transport to increasing levels of care for definitive operative treatment.

The microbiology of war wounds has changed as medicine and warfare have evolved. It is intriguing that within the current US military casualty system the microbiology of war wounds may be unique depending on the echelon of care and the time from injury. Previous reports of bacterial isolates in casualties from current combat actions in Iraq and Afghanistan showed the varying levels of microbiology laboratory support based on the echelon of care and the time of injury.[1–5] The variation in bacteriology laboratory capabilities involves specimen collection and culture procedures. In particular, bacterial quantification of wound tissue is not routinely available. To most accurately determine the microbiology of war wounds in US casualties, specimen collection and quantitative culture analysis must be standardized and assessed against time-specific clinical management of patients at each echelon of care.

Bacterial quantification of tissue specimens has been shown to be a useful adjunct in the management of open wounds and the timing of delayed wound closure or coverage, with bacterial loads greater than 1×10^5 colony-forming units per gram (CFU/g) of tissue representing a clinically relevant threshold differentiating colonization and contamination from critical contamination and infection.[6] Levels of tissue bacterial load above or below this threshold are associated with an increased incidence of delayed wound healing and successful skin grafting, respectively. The limit of detection of swab cultures exceeds significantly that of quantitative bacteriology of tissue biopsy samples, resulting in an relative underestimation by 1 to 2 logs.[7–12] With CFU levels $<1 \times 10^5$/g, wound closures have been reported to be 96% successful, whereas when tissue CFU levels are $>1 \times 10^5$ success decreases to a reported 20% skin graft survival.[13,14]

The present study was designed to determine by quantitative bacteriology the microbial flora of present-day war wounds in US troops from Iraq and Afghanistan upon arrival to and initial hospitalization at the National Naval Medical Center (NNMC), a tertiary care hospital (echelon 5). NNMC is the primary receiving hospital for all Navy and Marine Corps casualties, in addition to being the primary receiving hospital for all casualties (Army, Air Force, Navy, and Marines) with penetrating head injuries. To determine the microbiology of war wounds in the most clinically meaningful and objective fashion, quantitative microbiology of sequential wound tissue biopsies was performed.

Methods

The NNMC receives casualties directly from Landstuhl Regional Medical Center. From January 2007 through September 2008, casualties arriving at NNMC were eligible for enrollment into a NNMC Institutional Review Board–approved protocol to study combat wound biomarkers. Standard clinical practice at NNMC is to use vacuum-assisted wound closure devices (VAWCDs) in all open combat wounds anatomically amenable to VAWCD placement as the initial treatment; this includes both clinically clean and dirty wounds. Eligible patients were adult male and female military health care beneficiaries with extremity wounds sustained in the theater of operations (including the shoulder and buttocks without communication to the thoracic or abdominal cavities) treated with repeated surgical and VAWCD wound debridement and wound bed preparation. Patients with confounding comorbid conditions such as immune disorders, connective tissue disorders, or any conditions requiring immunosuppressive agents were not eligible. Up to 3 wounds maximum per patient were evaluated. In patients with more than 3 eligible wounds, the largest were evaluated. Wounds were ultimately closed by either delayed primary closure or a split-thickness skin graft at the discretion of the patient's attending surgeon.

Demographic variables, current antibiotics, and injury specifics were reviewed and recorded at the time of arrival. Upon arrival to NNMC, clinical parameters and wound characteristics were recorded daily with APACHE II scoring performed immediately before surgical wound care. Wounds were photographed and dimensions measured before each surgical debridement using digital planimetry and computerized digital management. Surgical debridement, pulse lavage, and VAWCD reapplication were performed every 48 to 72 hours until wound closure or coverage according to current institutional standards of practice. Only black VAWCD sponges were used during the study. Wound surface area (cm^2) and volume (cm^3) were calculated using PictZar planimetry software (BioVisual Technologies, Elmwood Park, NJ).

During each debridement, a 1-cm^3 wound tissue specimen was obtained from the center of the wound bed,

Table 1 Patient demographics

Age			
Median	21	Range 18–42	
Sex			
Male	34		
Female	0		
Injury Severity Score			
Mean	19.8	Range 8–45	SD: ±11.7
APACHE II score (at arrival)			
Mean	6.6	Range 1–26	SD: ±5.0
Wounds per patient			
1	23 patients	67.6%	
2	9 patients	26.5%	
3	2 patients	5.9%	
TBI			
Yes	28	6	
No	82.4%	17.6%	
Time from injury to arrival at NNMC			
Mean	5.6 d	Range 2–12 d	SD: ±2.7
Theater of injury			
OIF	26	8	
OEF	76.5%	23.5%	
Mechanism of injury			
Blast	30	4	
Other	88.2%	11.8%	
Tobacco use			
Yes	10	24	
No	29.4%	70.6%	
BMI			
Mean	25.094	Range 18–35.9	SD: ±3.89

weighed, and stored in a sterile 15-mL conical vial. The tissue specimens were transferred to a sterile, disposable tissue grinder; diluted 1:10 (wt/vol) in fastidious broth to a final concentration of 0.1 gram of tissue per milliliter; and ground until fully homogenized. Homogenized tissue specimens (1 μL and 10 μL) were inoculated on sheep's blood agar and MacConkey plates in triplicate. Plates were incubated overnight at 37°C. After incubation, colonies were counted, and the number of CFUs per gram of tissue was determined. Phenotypic identification of colonies was accomplished using Phoenix (BD) automated bacterial identification system (Becton Dickinson, Sparks, MD).

Results

Thirty-five patients were enrolled in the study from January 2007 to September 2008; all patients were male. One patient enrolled died shortly after arrival to our institution secondary to his injuries; 34 patients were fully evaluable and used for data analysis. Twenty-three patients had 1 wound, 9 patients had 2 wounds, and 2 patients had 3 wounds for a total of 54 wounds. All patients were male with a median age of 21 years with a mean Injury Severity Score and arrival APACHE II scores of 19.8 ± 11.2 (range 1–43) and 6.6 ± 4.7 (range 1–26), respectively. Twenty-eight patients had evidence of traumatic brain injury, representing an incidence of 82.4%. The mechanism of injury was predominantly blast (88.2%), and most patients had been injured in Iraq (76.5%) (Table 1). Wound debridement/treatment and antibiotic data were available for echelon 4 care; however, pre–echelon 4 data were inconsistently available/verifiable. The mean number of echelon 4 debridements and the number of antibiotics administered before arrival to the NNMC was 2.4 (range 1–3) and 2 (range 0 – 6), respectively. No pre–echelon 5 wound biopsies for quantitative culture were preformed.

Fifty-four wounds were studied and included in the analysis. Forty-two wounds (77.8%) were in the lower extremities and 12 in the upper extremities (22.2%). The mean surface area of the wounds was 239.5 cm^2 (standard deviation [SD] ± 270.6 cm^2, range 25.1–1,729.2 cm^2). The mean wound volume was 369.5 cm^3 (SD ± 298 cm^3, range 1.5–2,118.8 cm^3). Two hundred forty-two tissue biopsies and cultures were performed on the 54 wounds. The mean number of biopsies per wound was 3.2 (SD ± 2.5).

Of the 242 biopsies, 167 biopsies (69.0%) showed no growth on culture, and 75 (31.0%) were positive for bacterial growth. *Acinetobacter baumannii* was the most prevalent isolate; it was identified in 55 biopsies (22.7%) and accounted for 63.0% of all bacterial isolates. The second and third most prevalent bacterial isolates were *Enterococcus faecium* (10 biopsies, 4.1%) and *Escherichia coli* (6 biopsies (2.5%). Additionally, *Achromobacter species* (4%, 1.6%), *Enterobacter cloacae* (2%, 0.8%), *Pseudomonas stutzeri* (2%, 0.8%), *Staphylococcus aureus* (2%, 0.8%), *Staphylococcus haemolyticus* (2%, 0.8%), *Citrobacter freundii* (2%, 0.8%), *Alloicoccus otitis* (1%, 0.4%), and *Bacillus cereus* (1%, 0.4%) were isolated from tissue biopsies (Table 2).

Ninety-one of the 242 wound biopsies were obtained within 1 week of injury, 102 were obtained during the second

Table 2 Cumulative biopsy results

	Number of biopsies	Frequency
No growth	167	69.0%
Growth	75	31
Isolates	**Number of biopsies**	**Incidence (all biopsies/+ biopsies) (%)**
A baumannii	55	22.7/63
E faecium	10	4.1/12
E coli	6	2.5/7
A species	4	1.7/5
E cloacae	2	0.8/2
P stutzeri	2	0.8/2
S aureus	2	0.8/2
S haemolyticus	2	0.8/2
C freundii	2	0.8/2
A otits	1	0.4/1
B cereus	1	0.4/1

Table 3 Wound culture biopsy results by time period

Wound culture biopsy results	1st week postinjury (n = 91) (%)	2nd week postinjury (n = 102) (%)	>3rd week postinjury (n = 49) (%)
No growth	66 (72.5)	70 (68.6)	31 (63.3)
Any growth	25 (27.5)	32 (31.4)	18 (36.7)
Polymicrobial growth	7 (7.7)	3 (2.9)	1 (2)
bacteria isolates			
Total number of isolates	33	35	19
A baumannii	20 (60.6)	24 (68.5)	11 (57.9)
P stuzeri	2 (6.1)	0 (0)	0 (0)
E faecium	3 (9.1)	6 (17.1)	1 (5.3)
E Coli	1 (3)	0 (0)	5 (26.3)
E species	2 (6.1)	0 (0)	0 (0)
A species	3 (9.1)	1 (2.9)	0 (0)
B cereus	1 (3)	0 (0)	0 (0)
A species	1 (3)	0 (0)	0 (0)
S aureus	0 (0)	2 (5.7)	0 (0)
C freundii	0 (0)	2 (5.7)	0 (0)
S haemolyticus	0 (0)	0 (0)	2 (10.5)

week postinjury, and 49 occurred more than 2 weeks after injury. The incidence of any bacterial isolation from biopsies weekly was 27.5%, 31.4%, and 36.7%, respectively. Polymicrobial tissue cultures occurred at lower incidences, 7.7%, 2.9%, and 2% (Table 3) Throughout all time periods, *Acinetobacter* was the most common bacterial isolate cultured, representing 20 of the 33 isolates cultured in the first week postinjury (60.6%), 24 of the 35 isolates cultured during the second week postinjury (68.5%), and 11 of the 19 bacterial isolates greater than 2 weeks postinjury (57.9%) (Table 3)

The incidence of *Acinetobacter* isolation from a wound biopsy during the first week postinjury was 22.0%, 23.5% the second week, and 22.5% 3 or more weeks after the injury. The next most frequent isolates on wound biopsy during the first week postinjury were *E faecium* (3.3%), *A species* (3.3%), *P stutzeri* (2.2%), *E cloacae* (2.2%), and of lesser incidence *E coli* (1.1%), *B cereus* (1.1%), and *A otitis* (1.1%). These reported incidences are inclusive of polymicrobial growth; hence, the incidence of bacterial isolation (growth positive) is higher than the incidence of bacterial growth-positive wounds (Fig. 1).

During the first week postinjury, 27 patients had wound biopsies taken and cultured. The incidence, on a per-patient basis, of single-organism isolation was as follows: *A baumannii*, 14.8% (n = 4), *P stutzeri*, 4.3% (n = 1); *E faecium*, 4.3% (n = 1); and *E coli*, 4.3% (n = 1). Four patients showed polymicrobial growth during the first week postin-

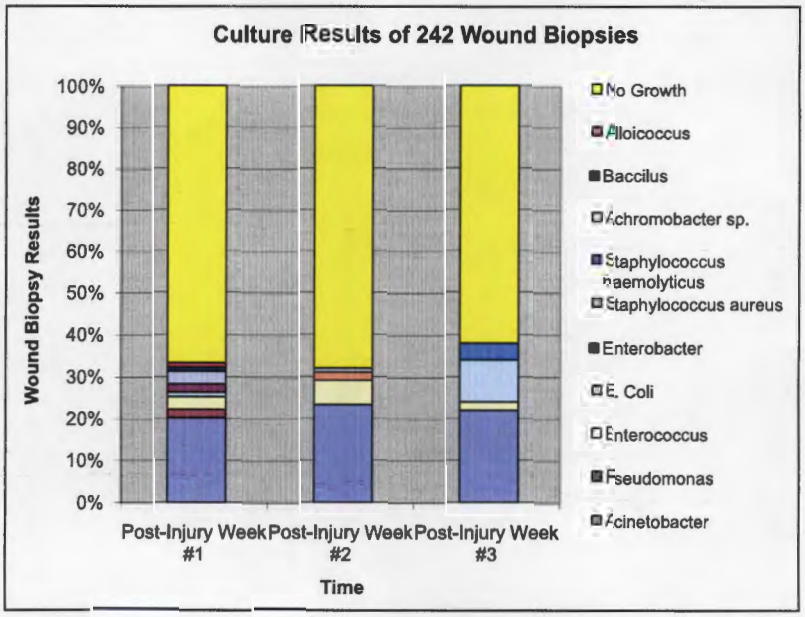

Figure 1 The frequency of isolates in 242 wound biopsies by time period.

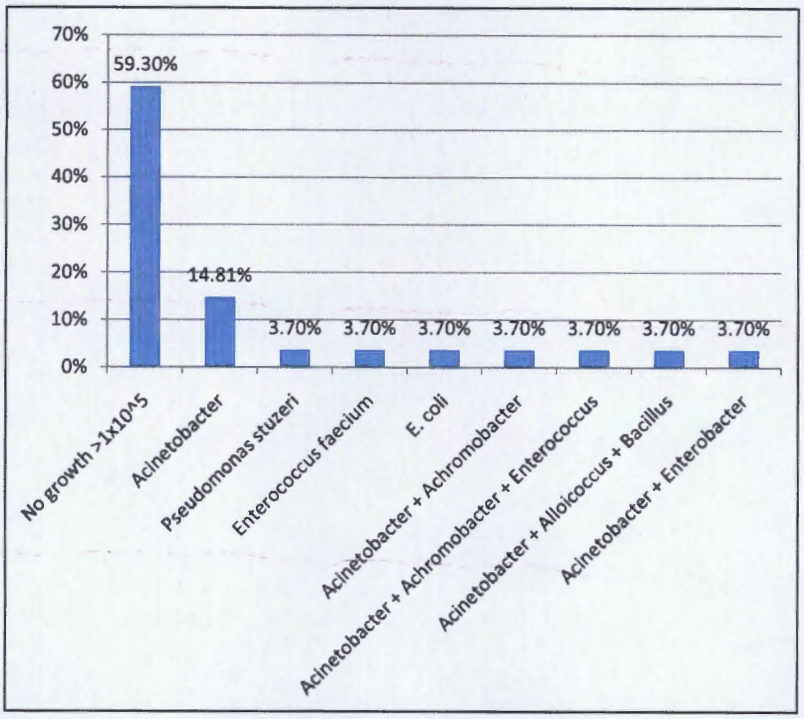

Figure 2 The first-week postinjury patient (n = 27) incidence of bacterial isolates > 1 × 10^5 CFU/g of tissue.

jury including the following: *Acinetobacter* + *Achromobacter*, 3.7% (n = 1); *Acinetobacter* + *Enterobacter*, 3.7% (n = 1); *Acinetobacter* + *Achormobacter* + *Enterococcus*, 3.7% (n = 1); and *Acinetobacter* + *Alloicoccus* + *B cereus*, 3.7% (n = 1). This was the only time period in which *Alloicoccus* and *B cereus* were isolated, and this occurred in the context of polymicrobial growth in 1 patient. These results represent isolates with greater than 1 × 10^5 CFU/g; there was only 1 culture growth isolate that occurred with less than 1 × 10^5 CFU/g during this time period, and that isolate was *Acinetobacter* (Fig. 2).

During the second week postinjury, 28 patients had wound biopsies taken and cultured. The incidence, on a per-patient basis, of single-organism isolation was as follows: *A baumannii*, 14.3% (n = 4); *S aureus*, 3.6% (n = 1); *C freundii*, 3.6% (n = 1); *E faecium*, 3.6% (n = 1); and *Achromobacter*, 3.6% (n = 1). Two patients showed the following polymicrobial growth during the second week postinjury: *Acinetobacter* + *Enterobacter*, 3.6% (n = 1), and *Acinetobacter* + *Enterococcus*, 3.6% (n = 1). These results represent isolates with greater than 1 × 10^5 CFU/g. Fifteen patients had no growth during the second week, and 3 patients grew the following isolates with less than 1 × 10^5 CFU/g during this time period: *Acinetobacter* alone (n = 1), *Enterococcus* alone (n = 1), and *Acinetobacter* + *Enterococcus* (n = 1) (Fig. 3).

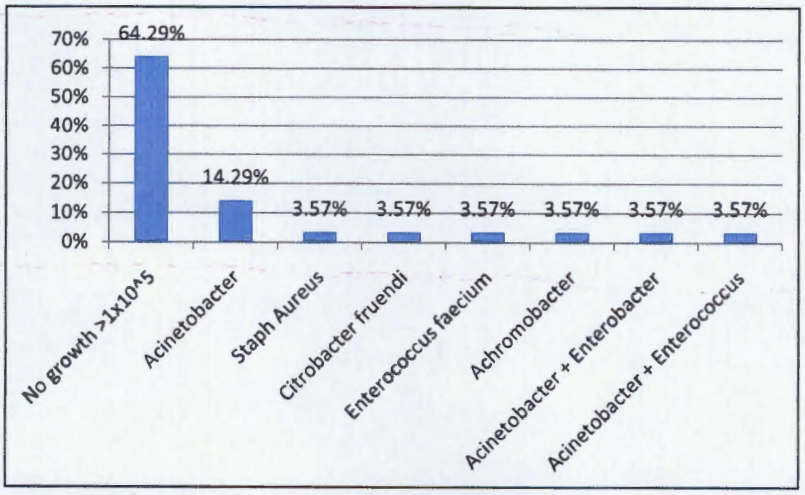

Figure 3 Second-week postinjury patient (n = 28) incidence of bacterial isolates >1 × 10^5 CFU/g.

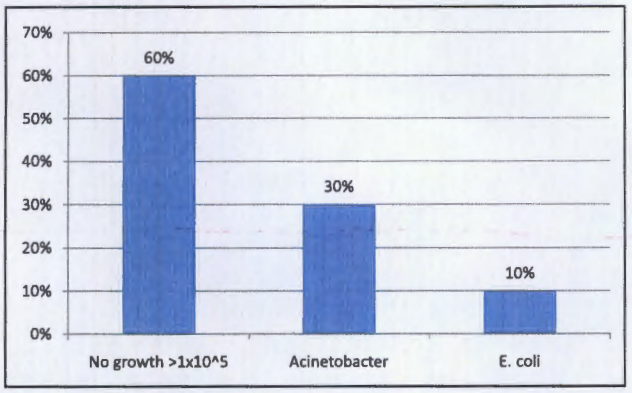

Figure 4 Third-week postinjury and later patient (n = 10) incidence of bacterial isolates $>1 \times 10^5$ CFU/g.

After the second week postinjury, 10 patients had wound biopsies performed and cultured. The incidence, on a per-patient basis, of *A baumannii* was 30.0% (n = 3), and for *E. coli* it was 10.0% (n = 1). These results represent isolates with greater than 1×10^5 CFU/g. No patients showed polymicrobial growth or isolated with less than 1×10^5 CFU/g beyond the second week postinjury (Fig. 4).

Comments

In the present study, most wound biopsy cultures were obtained within the first 2 weeks after injury, which is consistent with most wounds being closed within that timeframe. Most wounds underwent multiple biopsies during a given period, and additionally one third of patients had multiple wounds. The analysis of wound microbiology based solely on a biopsy basis could therefore be misleading and justifies analysis on a by-patient basis. Most cultures showed no growth regardless of the time period studied, and the incidence of polymicrobial and colonized ($<1 \times 10^5$ CFU/g) cultures was unexpectedly low and may reflect the effectiveness of the frequent and aggressive measures undertaken with regards to wound and global casualty treatment at and before echelon 5 treatment. The wound biopsies did not appear to compromise wound healing in any way.

Gram-negative bacteria predominated throughout the study, with *A baumannii* being the most common pathogen at all time points by both individual biopsy and by patient analysis. The observed gram-negative and *A baumannii* predominance is consistent with previous reports of echelon 5 microbiology in orthopedic wounds and osteomyelitis isolates.[1,2] However, the current report is unique in that the *A baumannii* and gram-negative predominance is temporally documented as being present upon arrival to echelon 5 care with decreasing prevalence during the hospitalization and therefore not a result of nosocomial transmission or antibiotic selection at the echelon 5 facility.

Reports of pre–echelon 5 microbiology during the current US conflicts are mixed and indicate the importance of US-specific casualty analysis and a potential effect of the early echelons' mixed nationality casualty populations. The USNS COMFORT (echelon 3) deployment to the Persian Gulf in 2003 documented an overall propensity of gram-negative isolates, with *A baumannii* being the predominant wound isolate in clinically infected wounds (34%). However, specimen attainment methods and quantified microbiology were not specified, and analysis included both US and Iraqi national patients. Fifty-six patients were included in the study; however, during the study period, a total of 211 patients were treated. Eighty-five percent were Iraqi nationals, and 35% were US casualties with no stratification as to the incidence of bacteriology by nationality.[3] Additionally, reports from an echelon 3 treatment facility in Baghdad showed an early gram-positive predominance in US troops in contrast to a gram-negative predominance in non-US casualties.[4,5] The results of the present study and these previous reports support the concept of a change in the bacteriology of US wounds based on nosocomial transmission at the echelon 3 level of care possibly in conjunction with the present practice of empiric broad-spectrum antibiotic administration in the treatment of US casualties.

The current study is unique compared with previous reports of microbiology during this conflict on several accounts. The time from injury to each culture was documented, and the method of specimen attainment for culture was standardized and microbiological quantification used. Additionally, the results reflect not only the flora acquired at echelon 5 but also are reflective of pre–echelon 5 care.

The microbiology of US casualty soft tissues may vary as casualties pass through the current military medical system from combat theater and echelon 4 facilities to echelon 5 care. The importance of the present report is the wound microbiology encountered upon arrival to and initial treatment at CONUS echelon 5 care. The fact that most US casualties do not have significant levels ($>1 \times 10^5$ CFU/g) of wound bacteria at the time of arrival to echelon 5 care and that these levels of wound microbes decrease during the hospitalization potentially support a shift away from the current echelon 5 practice of continuing arrival empiric wound antibiotics and planned delayed wound closure only after multiple debridements to one of limited empiric wound antibiotic use and more rapid wound closure. However, the present study is limited in that pre–echelon 5 variables (ie, microbiology, wound care, antibiotic use, and geography) are insufficient to permit analysis; such analysis may permit the determination of wounds amenable to early closure and those best treated by multiple debridements and delayed closure at arrival to echelon 5 care. The initial microbiology of pre–echelon 5 care and the microbiology of US casualty soft-tissue war wounds throughout the evacuation chain may or may not vary depending on the level of care. This possibility should be further investigated using consistent, standardized, and most clinically relevant methodologies to ensure comparability and clinically relevant microbial quantification. Such studies are needed to more completely un-

derstand the microbiology of US casualty combat wounds and the effectiveness and effect of care at all levels of care and further improve the care of our casualties.

Acknowledgment

The multidisciplinary care of these patients would not have been possible without the dedicated efforts of everyone at National Naval Medical Center (NNMC). Both civilian and military personnel have rendered skilled and compassionate care for these casualties. All of our efforts are dedicated to those who have been placed in harm's way for the good of our nation.

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